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SHORT COMMUNICATION

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Soil amidase activity in polyacrylamide-treated soils and potential activity toward common amide-containing pesticides

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Abstract Polyacrylamide (PAM) is currently used as an irrigation water additive to significantly reduce the amount of soil erosion that occurs during furrow irrigation of crops. Elevated soil amidase activity specific toward the large PAM polymer has been reported in PAM-treated field soils; the substrate specificity of the induced amidase is uncertain. PAM-treated and untreated soils were assayed for their capacity to hydrolyze the amide bond in carbaryl (Sevin), diphenamid (Dymid), and naphthalene acetamide. Based on results obtained with a soil amidase assay, there was no difference between PAM-treated and untreated soils with respect to the rate of amide bond hydrolysis of any of the agrochemicals tested. It appears that under these assay conditions the PAM-induced soil amidase is not active toward the amide bonds within these molecules. However, carbaryl was hydrolyzed by a different soil amidase. To our knowledge, this is the first soil enzyme assay-based demonstration of the hydrolysis of carbaryl by a soil amidase.

Key words Polyacrylamide · Soil amidase · Carbaryl · Diphenamid · Naphthalene acetamide

Introduction

Polyacrylamide (PAM) is currently added to agricultural soils as an extremely efficient anti-erosion additive in irrigation water (Lentz and Sojka 1994). This PAM has a very high molecular weight ($1–2 \times 10^7$) and a linear, anionic configuration. It is made up of approximately 82 mol% acrylamide subunits and 18 mol% acrylate subunits (Lentz et al. 1992). The resulting polymer consists of repeating ethylene units in the backbone, with amide and carboxylic acid substituents as side chains (Fig. 1A).

We have established bacterial enrichment cultures from agricultural soil, which are able to gain N benefit from PAM (Kay-Shoemake et al. 1998a). The amidase produced in these cultures hydrolyzed the PAM polymer as well as two small amides, formamide and propionamide.

Amidase activity in the soil environment has been described in detail by Frankenberger and Tabatabai (1980a,b, 1981a,b, 1985). Generally, soil enzyme activity is thought to derive from secreted extracellular enzyme or enzyme released upon cell lysis, which becomes immobilized in an active form on soil particles (Burns 1982; Ladd 1985; Nannipieri 1994; Skujins 1976). It has been documented that the acrylamide monomer is subject to decomposition in soils; this transformation is assumed to be due to amidase activity (Abdelmagid and Tabatabai 1982).

Amidase activity has been associated with the degradation of numerous agrochemicals in laboratory experiments (Bollag and Liu 1990; Steen and Collette 1989). Field studies indicate that PAM-treated agricultural soils exhibit elevated soil amidase activity that is specific for the PAM polymer (Kay-Shoemake et al. 1998b). It is unclear from the laboratory and field data whether the PAM-induced amidase activity is indicative of a single amidase with broad specificity or of a group of enzymes, each with different amide specificity. PAM contains unsubstituted amide-N groups, but it is not known if the amidase that hydrolyzes PAM is capable of acting on substituted amide bonds or on arylamide substrates. Three arylamide pesticides were selected for study based on the degree of substitution at the amide-N position (Fig. 1B). This study was conducted to determine if these amide-containing agrochemicals would undergo more rapid enzymatic hydro-

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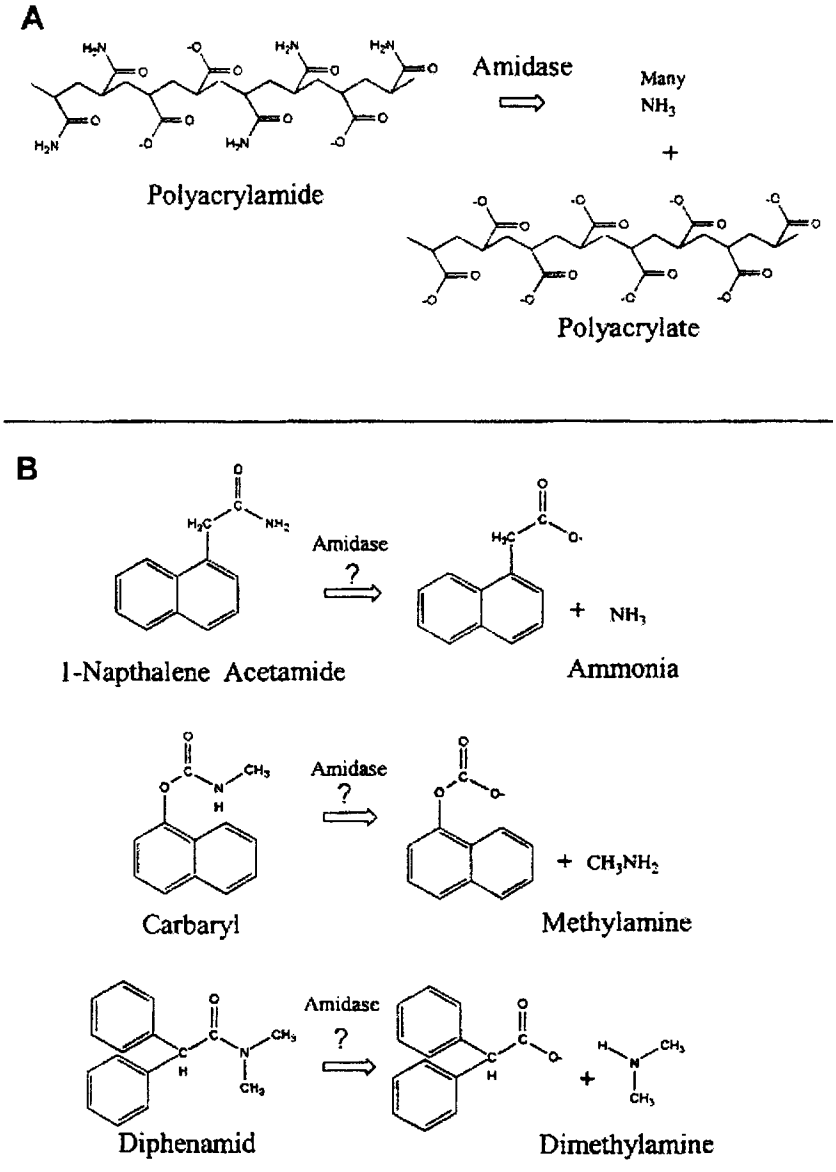

Fig. 1 Molecular structures of polyacrylamide (**A**) and the amide-containing pesticides (**B**) used as substrates in the soil amidase assays

lysis in soil which had been treated with PAM and exhibited elevated PAM-specific amidase activity.

Materials and methods

Site description and soil sampling

Soils were collected from PAM-treated and untreated soil microcosms maintained in the Department of Biological Sciences greenhouse facility at Idaho State University. Each microcosm consisted of a polypropylene box (25 cm deep × 52 cm long × 37 cm wide) filled to a depth of 20 cm with Portneuf silt loam A horizon soil (coarse-silty, mixed, mesic, Durixerollic Calciorthid), of approximate pH 7.8, and 2–8% $CaCO_3$ equivalent. Two furrows, formed in each microcosm, were 9 cm wide and 52 cm long, with 8 cm between-furrow spacing. The microcosms were planted to green beans (var. Blue Lake), and each furrow was irrigated every 2 weeks with 10 l of either PAM-treated tap water (10 mg l^{-1}) or untreated tap water. The flow rate was 7.6 l min^{-1} The microcosms received this treatment for approximately 1 year prior to the experiment.

Soils, collected from the upper 3 cm of each sampled furrow bottom, were assayed for PAM-specific amidase activity by a previously described procedure (Kay-Shoemake et al. 1998b), which is a modification of an assay originally described by Frankenberger and Tabatabai (1980a). The PAM-treated microcosm soil exhibited elevated PAM-specific amidase activity; untreated soil showed negligible activity. This pattern was also observed in field soils (Kay-Shoemake et al. 1998b).

Chemicals

The PAM used in microcosm applications was Superfloc 836A, provided by Cytec Industries (Stamford, Conn.); the polymer is described in detail by Lentz and Sojka (1994).

The pesticides used in the study represent a range of amide structural types. Carbaryl (2 amide; Sevin), diphenamid (3 amide; Dymid), and naphthalene acetamide (1 amide) were obtained from ChemService (West Chester, Pa.), all at purity >98%. All other chemicals were purchased from Fisher Scientific (Pittsburgh, Pa.) and were reagent grade.

Soil amidase assay

A modification of the method described by Frankenberger and Tabatabai (1980a) was used to measure soil amidase activity with amide-containing agrochemicals as substrate amides. Each test was conducted in triplicate. Briefly, PAM-treated or untreated soils (5 g) were exposed to toluene (0.2 ml) in a 225-ml wide-mouth polypropylene jar. Each agrochemical, dissolved in acetone to yield a concentration of 67 mM, was applied (15 µl) separately to the toluene-treated soil. Ten milliliters of THAM (Tris-(hydroxymethyl)amino methane) buffer (0.1 M, pH 8.5) was added to the assay mixture; jars were then capped and incubated at 37 °C for 24 h. Following incubation, 25 ml deionized water acidified with HCl (pH 3) was added to each test mixture, yielding a slurry, pH 6.5. Each slurry was agitated for 30 min then allowed to settle for 15 min. Supernatant samples were filtered, diluted 1:40 in acidified deionized water (pH 3), and analyzed for amidase hydrolysis products via ion chromatography. Upon amide hydrolysis carbaryl yields methylamine, diphenamid yields dimethylamine, and naphthalene acetamide yields ammonium (Fig. 1B).

Control assays were conducted as above, in triplicate, except that only toluene and buffer were added prior to incubation; no pesticide was added at any point. An additional control consisted of diluted pesticide, in which the quantity of hydrolysis product was determined in the absence of amidase activity. The amounts of hydrolysis product detected in these two controls were subtracted from that detected in each test mixture derived from enzymatic hydrolysis.

An additional set of control assays consisted of toluene-treated soil (PAM treated or non PAM treated) to which acetone was added (no agrochemical was present in the acetone) and incubated for 24 h. These controls were compared to toluene-treated soils without acetone with respect to the amount of extractable ammonium, methylamine, and dimethylamine. The addition of acetone did not affect levels of hydrolysis products present in either PAM-treated or non-PAM-treated soil (data not shown). Preliminary experiments indicated that acetone did not interfere with soil amidase activity.

Analytical techniques

Dimethylamine, methylamine, and ammonium were quantified using a Dionex 100 ion chromatograph equipped with a CS12 cation detection column, self regenerating suppressor, and a conductivity detector. Methane sulfonic acid (MSA; 20 mM) was the eluent; the flow rate was 1.0 ml min^{-1}. Detection limits for dimethylamine, methylamine, and ammonium were 0.5 mg l^{-1}, 0.1 mg l^{-1}, and 0.01 mg l^{-1}, respectively, and extraction efficiencies were 94%, 98%, and 93%, respectively.

Results and discussion

Hydrolysis due to soil amidase activity was observed in the case of carbaryl; no spontaneous hydrolysis of the carbaryl-only preparation was observed (Table 1). However, there was no significant difference between the PAM-treated soil and the untreated soil. For naphthalene acetamide and diphenamid, hydrolysis was observed, but hydrolysis appeared to be spontaneous and non-biological. Levels of hydrolysis products measured for these substrates in the absence of soil were not different from levels determined in the complete soil assay mixtures (Table 1).

Exposure of the soils to toluene could be construed as problematic, since toluene has been cited as exhibiting inhibitory activity toward aryl- and alkylamidases (Frankenberger and Tabatabai 1985). However, the observations that PAM-specific amidase activity remained intact in these soils and that carbaryl (an arylamide) apparently served as substrate for an amidase, albeit not the PAM-specific amidase, indicated that amidase activity was probably not inhibited by this chemical. Treatment with toluene, a bacterial growth inhibitor, was essential due to the highly metabolizable nature of the hydrolysis products. Furthermore, Frankenberger and Tabatabai's (1985) observations of inhibitory effect were made only for purified enzyme preparations; no such effect was noted for whole cell activity.

Apparently PAM-specific soil amidase does not hydrolyze the chemicals included in this study. This may be due to specific chemical characteristics. For example, the chemicals examined in this study are arylamides, as opposed to PAM, an alkylamide. It may be that the PAM-specific amidase (or suite of amidases), induced in the presence of PAM, is alkyl specific. Further work is necessary to assess the roles of other factors, including degree of N substitution, the degree of carbonyl substitution, and relative hydrophobicity of neighboring groups, in defining substrate specificity of the PAM-active amidase.

The soil amidase assay used in these investigations has previously only been used on water-soluble substrates (Frankenberger and Tabatabai 1980a,b; 1981a,b; 1985). The hydrophobic nature of the pesticides may have confounded the assay, but all of the hydrolysis

Table 1 Hydrolysis product (µg g^{-1} soil) detected in the amidase assays[a]. Each value represents the mean of triplicate determinations ±1 SE. *PAM* Polyacrylamide

Incubation mixture	No soil	PAM-treated soil	Untreated soil
Carbaryl only	0		
Soil + buffer only		0	0
Soil + buffer + carbaryl		138.7 ± 0.1	145.7 ± 0.2
Naphthalene acetamide only	40.9 ± 1.0		
Soil + buffer only		22.3 ± 0.6	17.2 ± 0.2
Soil + buffer + naphthalene acetamide		39.1 ± 0.7	40.7 ± 1.1
Diphenamid only	18.7 ± 0.9		
Soil + buffer only		0	0
Soil + buffer + diphenamid		29.6 ± 1.2	27.7 ± 0.7

[a] Hydrolysis products for carbaryl, naphthalene acetamide and diphenamid were methylamine, ammonium and dimethylamine, respectively

products are water soluble and should have been detectable in the acidified extracts. Furthermore, amidase activity toward carbaryl was detected despite the limited solubility of the substrate (120 mg l^{-1}).

These data indicate that there is a soil amidase that hydrolyzes carbaryl, although it not the same soil amidase that hydrolyzes PAM. We are familiar with the cultivation history of this soil for the 2 years prior to the experiment; it is not known whether carbaryl exposure occurred previously.

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